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# Protein network reconstruction of CHO cell secretory pathway

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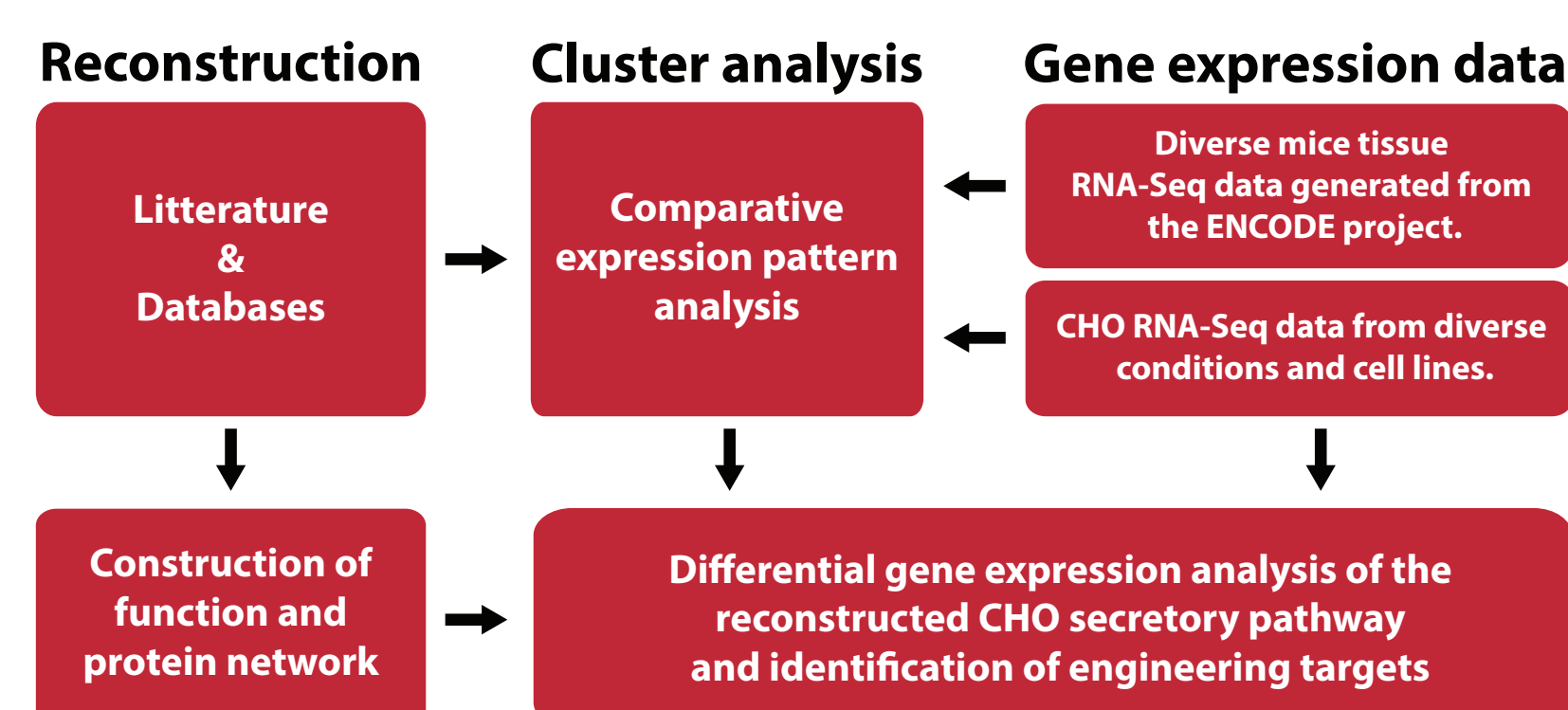
## Introduction

Protein secretion is one of the major bottlenecks in the productivity of recombinant protein in mammalian cells. So far, there have been limited studies of the cell biology of the CHO cell and the potential of cell line engineering. To elucidate the poorly understood cellular processes that control and limit recombinant protein production and secretion, a system-wide study was initiated to identify possibly engineering targets relevant for therapeutic protein production.

### Objective and Strategy

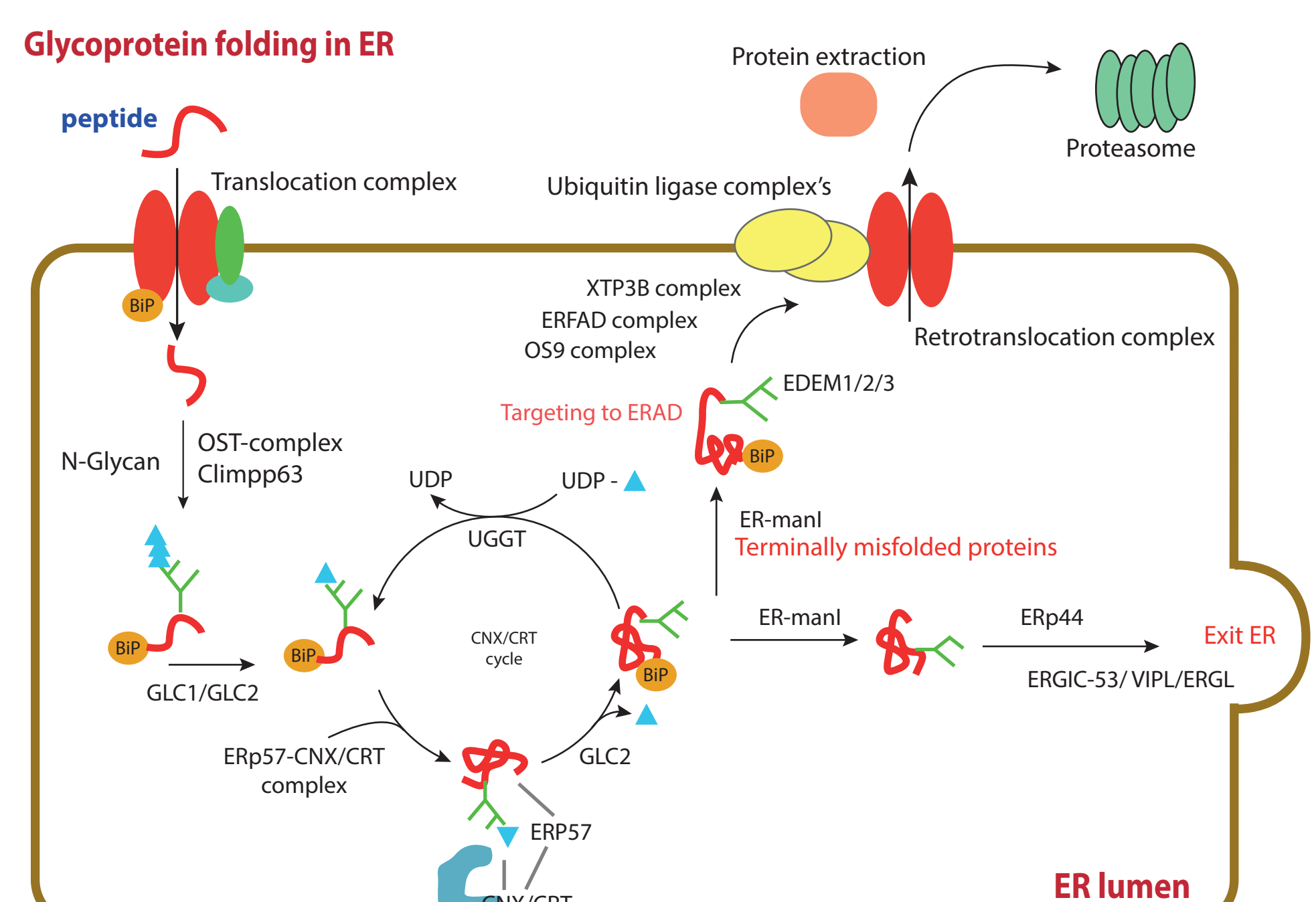
- Introduce a more systematic approach in improving the protein production in CHO cell lines
- Employ a guided approach that integrates protein function interaction network, gene expression and comparative studies of mouse and CHO cells.
- Identify functional gene targets within the secretory pathway for modification in order to increase protein production.

Workflow for reconstruction of CHO cell pathways:



### Pathway reconstruction

Proteins associated or linked to early secretion pathway were identified by manually curate available literature on mouse models and cell lines. The proteins found were used to identify CHO-K1 genes of the ERAD and protein folding pathway.



### RNA-Seq transcriptome data

RNA was extracted with TRIzol reagent under different growth conditions and treatments from diverse sets of CHO cell lines.

Samples:

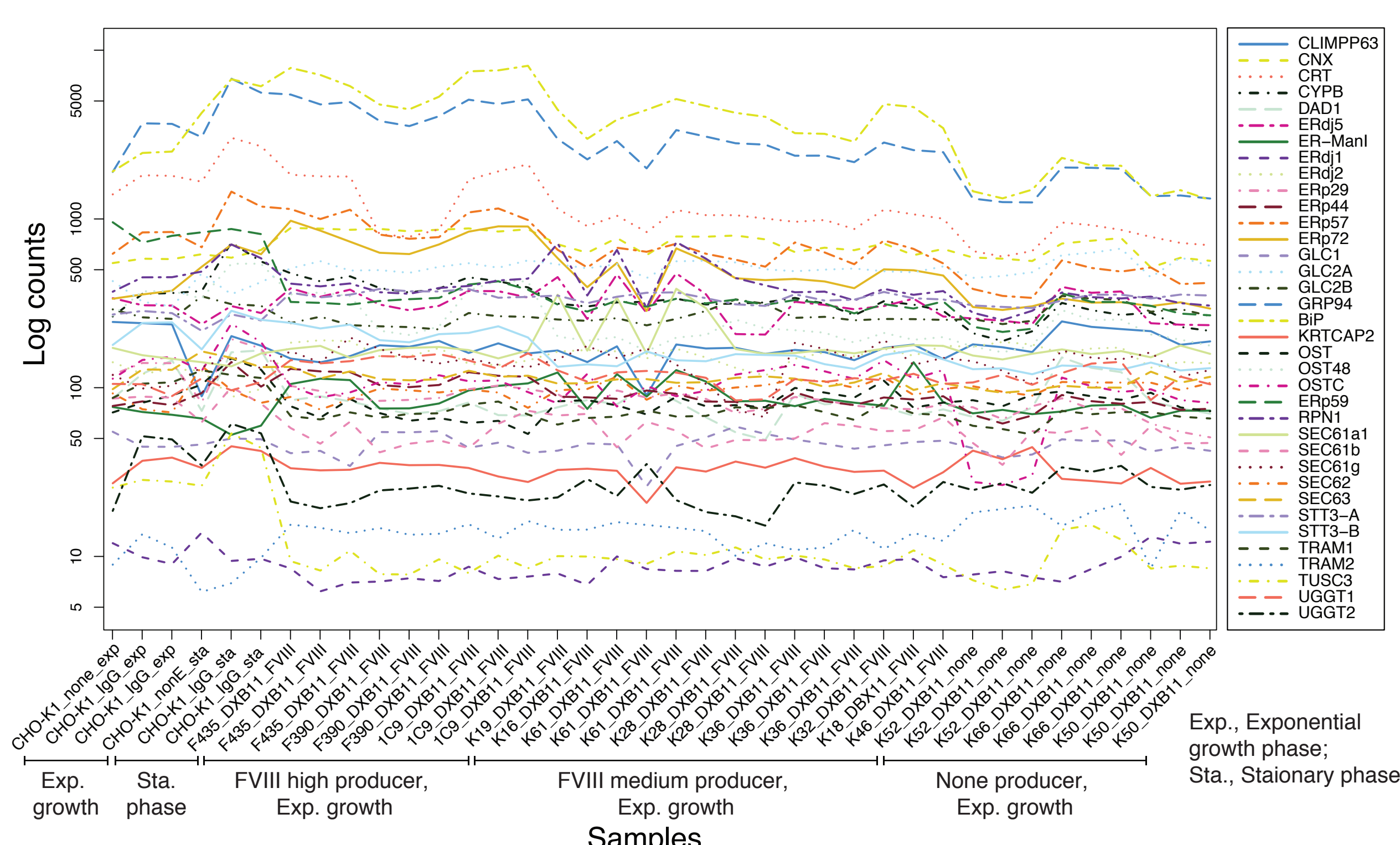
CHO-K1			CHO-DXB11		
exp. growth	stationary	NaBu	exp. growth		
IgG producer			FVIII producer		
None producer			high	medium	low

Library and paired-end RNA sequencing were performed by AROS a/s on Illumina HiSeq 2000 platform with a sequencing depth of min. 35 mio reads. Pre-processing and mapping of RNA-seq reads performed with Bowtie [1] and TopHat [2] using the CHO-K1 genome [3] as reference.

### Gene expression cluster analysis

A comparative expression analysis of CHO cells and mice allowed to evaluate CHO cell genes expression patterns and for identification of specific proteins and association with changed network arrangement in CHO cells.

#### CHO expression pattern protein folding associated genes

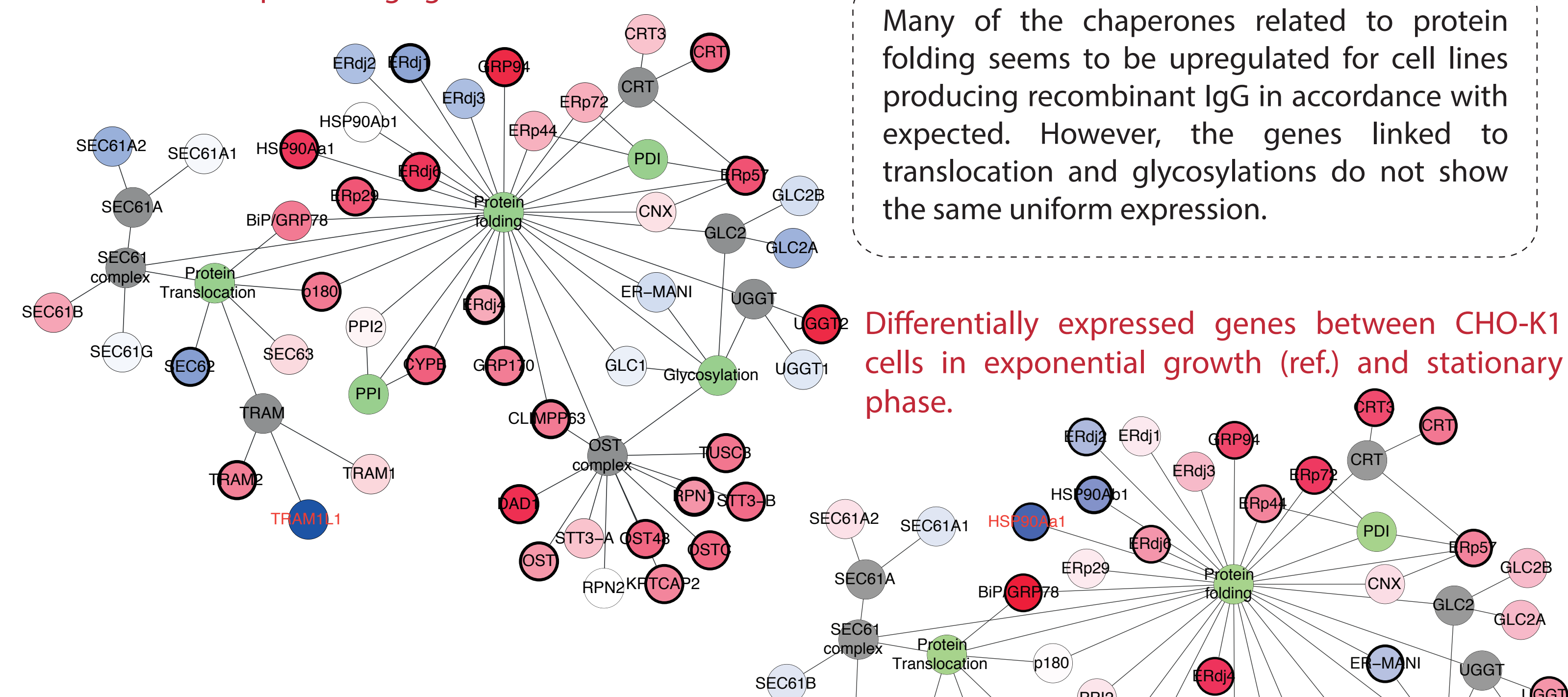


Hierarchical clustering by Spearman's correlation and average linkage. The robustness of the clustering was evaluated by bootstrapping using the R package pvclust nboot=1000.

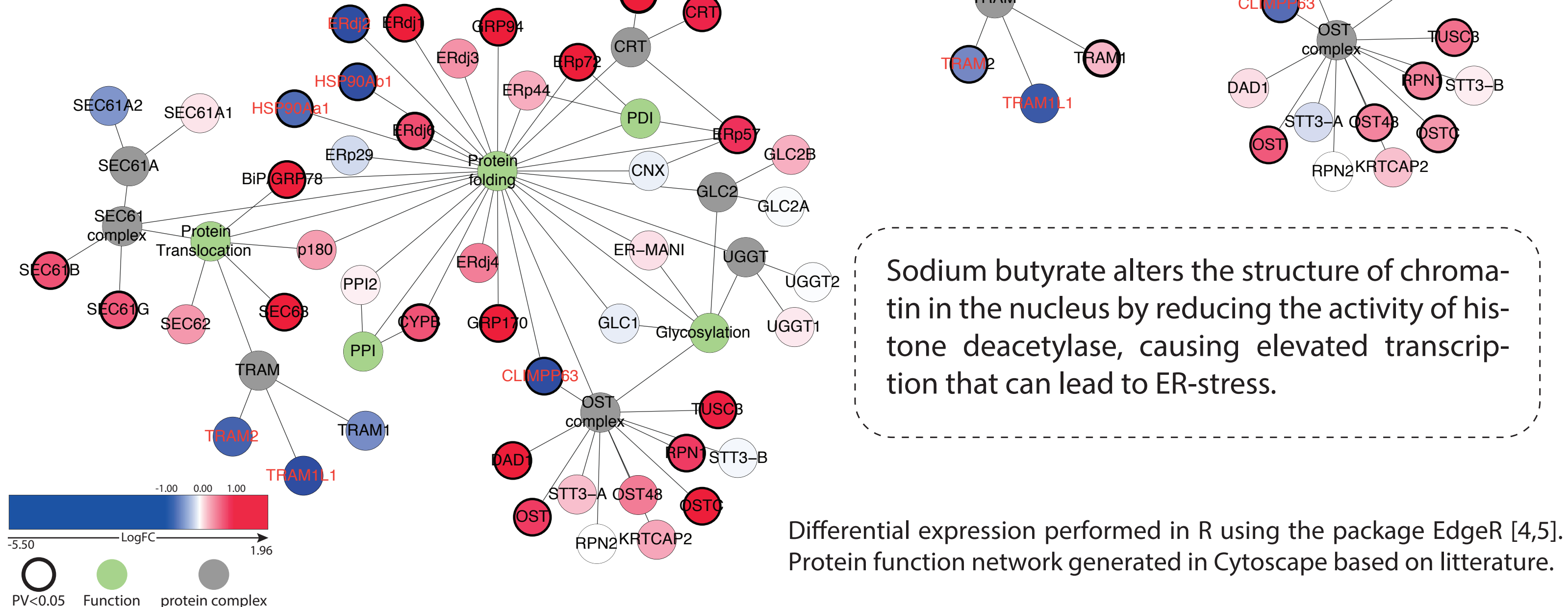
### Integrated gene expression network

The sub-network of genes associated with translocation and protein folding.

Differentially expressed genes between CHO-K1 cells (ref.) and CHO-K1 cells producing IgG.



Differentially expressed genes between CHO-K1 cells and CHO-K1 cells treated with NaBu.



### Conclusion

- This case study showed how a gene function interaction network and gene expression clusters may give insight to biological gene clusters and expression behaviour.
- Possible genetic targets can be evaluated in relation to the systemic network as well as identification of new targets.
- Identify changed regulation in functions or pathways within the secretory pathways.

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